ORIGINAL ARTICLE

Routine diagnosis of *Borrelia burgdorferi* (sensu lato) infections using a real-time PCR assay

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Objective To establish a one-tube fluorogenic real-time PCR assay for routine detection of *Borrelia burgdorferi* (sensu lato) DNA in various clinical specimens.

Methods A fragment of the flagellin gene sequence was amplified with the TaqMan chemistry using primers and a probe common to *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, *Borrelia garinii* and *Borrelia valaisiana*. A recombinant plasmid containing the chromosomal gene coding for the flagellin protein was used as standard.

Results The specificity of the assay was documented with 48 different clinically relevant *Borrelia burgdorferi* strains. No cross-reaction occurred with unrelated bacteria, viruses and fungi. At an analytic sensitivity of 10 copies, excellent precision within runs and between runs was observed. The potential presence of inhibitors of the *Taq* DNA polymerase was monitored by spiking aliquots of each sample with a plasmid containing the target sequence. Among 56 cerebrospinal fluid samples taken from 54 patients with clinical suspicion of neuroborreliosis, one (1.8%) tested positive for *Borrelia burgdorferi* sensu lato DNA. *Borrelia burgdorferi* DNA was also detected in five (17.9%) of 28 synovial fluid specimens and in one (20%) of five synovial membrane biopsies obtained from 31 patients with arthropathies. In order to test for the absence of false-positive results, 84 samples from 83 patients without evidence of Lyme disease were investigated. None of these samples showed measurable amounts of *Borrelia burgdorferi* DNA.

Conclusion By its established features, such as speed, reliability, sensitivity, specificity, the inclusion of carryover prevention and the monitoring of inhibitors in individual test tubes, this real-time PCR assay has proved to be a potent tool for the detection of *Borrelia burgdorferi* DNA under routine conditions in diagnostic laboratories.

Keywords *Borrelia burgdorferi*, real-time PCR, TaqMan, quantitative PCR, routine diagnosis, synovial fluid

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INTRODUCTION

Lyme disease is a multisystem infection caused by spirochaetes of the genospecies complex *Borrelia burgdorferi* sensu lato. Infection occurs through the bite of an infected tick of the Ixodidae family. Four *Ixodes* species are recognized worldwide as important vectors of Lyme borreliosis. These are *I. persulcatus* and *I. ricinus* in the old world, and *I. pacificus* and *I. scapularis* (*dammini*) in the new world.

In Europe, at least three species are known as causative agents of the disease: *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* and *Borrelia afzelii*. A fourth species, *Borrelia valaisiana* (group VS116), is still under consideration in this regard. *Borrelia valaisiana* has so far been associated with skin manifestations [1] and possibly with neurologic symptoms [2].

Despite the frequent appearance of a typical rash called erythema migrans (EM), the clinical manifestations are often not pathognomic, and usually a diagnosis cannot be made on clinical grounds alone. Rapid, specific and sensitive diagnosis of Lyme disease is required in order to provide patients with appropriate treatment.

Since the identification of *Borrelia burgdorferi* as the agent of Lyme disease, numerous serologic tests have been developed [3]. Unfortunately, such tests are prone to misdiagnosis of Lyme disease, despite the introduction of a two-step protocol including a second test (Western blot) to confirm a positive or indeterminate enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assay [4,5]. In particular, false-negative

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ELISA results may be recorded in patients with a diagnostic Western blot, thus limiting the validity of this assay format [6,7].

Diagnosis of Lyme disease may be established by cultivation of the pathogen in a special medium (Barbour, Kelly, Stoenner). However, the sensitivity of this technique is low, ranging from 30% to 70% for culture of skin biopsy specimens [8] to less than 5% for culture of cerebrospinal fluid (CSF) [9].

During the last decade, the PCR technology has been extensively used as an alternative diagnostic instrument for the detection of *Borrelia burgdorferi* DNA in clinical specimens [1,10–20]. In these studies, the PCR assay allowed the successful detection of *Borrelia burgdorferi* DNA in blood, CSF, urine, skin biopsy, synovial membrane and synovial fluid specimens obtained from selected categories of patients with Lyme disease. While those protocols illustrated the significance and use of PCR to complement or confirm the diagnosis of Lyme disease in research studies, their formats generally did not meet the requirements of a routine diagnostic laboratory.

In an attempt to overcome these limitations, we have developed a one-tube fluorogenic real-time PCR assay (TaqMan) for the detection of *Borrelia burgdorferi* (sensu lato) DNA in a routine laboratory setting. The test is based on the quantitative amplification of a fragment of the chromosomal gene coding for the flagellin protein. The potential presence of inhibitors is monitored using spiked specimens. A carryover prevention step together with the use of a closed-tube system minimize the risk of contamination. The characteristics of the assay have been evaluated using a large number of cultured strains as well as clinical specimens as substrates.

MATERIALS AND METHODS

Bacterial strains

Cultures of 48 isolates of the *Borrelia burgdorferi* (sensu lato) complex (12 *Borrelia burgdorferi* sensu stricto, 12 *Borrelia afzelii*, 11 *Borrelia valaisiana*, 13 *Borrelia garinii*) and 10 different unrelated *Borrelia* species were tested.

Clinical samples

Negative control group

Blood samples from 37 healthy blood donors and 47 samples (one urine, 34 CSF specimens, seven synovial fluid samples, two blood samples and three synovial membrane biopsies) from patients with unrelated infections were used as negative controls.

Patient specimens

These comprised 56 CSF samples from 54 patients with clinical suspicion of neuroborreliosis, and 33 samples (28 synovial fluids and five synovial membranes) from 31 patients with arthritis of unclear etiology.

Extraction of DNA

Total bacterial DNA was extracted from cultures and clinical specimens using a commercial kit (QIAamp DNA Mini Kit; Qiagen Corporation, Hilden, Germany). The supplier's protocol (QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook; protocol for bacteria and tissue 01/99) was optimized for recovery of low-copy-number DNA as follows. Suspension cultures, CSF, blood, synovial fluid or urine were centrifuged at 5000 *g* for 10 min, and the pellet was recovered in 180 μL of lysis buffer and 20 μL of proteinase K provided with the kit. The samples were incubated at 56 °C from 1 h to overnight. Synovial membranes were digested overnight at 56 °C in 180 μL of lysis buffer and 20 μL of proteinase K. Bound DNA was eluted twice in a total volume of 100 μL of elution buffer. The eluate was incubated at 95 °C for 5 min and chilled on ice until use. Ten microliters of the eluted DNA was used as template for the PCR.

Primers and probes

The published genome sequences of seven different isolates in the genus *Borrelia burgdorferi* sensu lato, *Borrelia afzelii* VS461 (D63365), *Borrelia afzelii* ACAI (X75202), *Borrelia afzelii* HT61 (D63366), *Borrelia burgdorferi* IP90 (L42885), *Borrelia burgdorferi* GeHo (X15660), *Borrelia burgdorferi* B31 (X15661) and *Borrelia garinii* HT22 (D63367), were selected from the taxonomy browser (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/), a database of the National Center of Biotechnology Information.

A multiple sequence alignment with Clustalw software was done on-line (http://bioweb.pasteur.fr/seqanal/interfaces/clustalw.html), to search for homology regions within the flagellin gene (Figure 1). The sequence of *Borrelia burgdorferi* B31 (X15661) was selected to search for primers and the fluorogenic TaqMan probe within a region of maximal homology using the computer program Primer Express 1.0 (Applied Biosystems, Foster City, CA, USA).

The forward primer FlaF1A, the reverse primer FlaR1 and the fluorogenic TaqMan probe FlaProbe1 were selected (Table 1 and Figure 1). The fluorescent dyes at the 5′ and at the 3′ ends of the probe are FAM (6-carboxyfluorescein; reporter) and TAMRA (6-carboxytetramethylrhodamine; quencher), respectively (Table 1). In addition, the absence of homology of the selected primers and the probe with unrelated sequences was checked with BLAST search, a computer program for sequence analysis of the NCBI (National Center of Biotechnology Information; http://www.ncbi.nlm.nih.gov/BLAST/).

The TaqMan fluorogenic probe was synthesized by Applied Biosystems, Weiterstadt, Germany. The primers were made at our institution with an ABI 392 DNA/RNA Synthesizer (Applied Biosystems, Foster City, CA, USA).

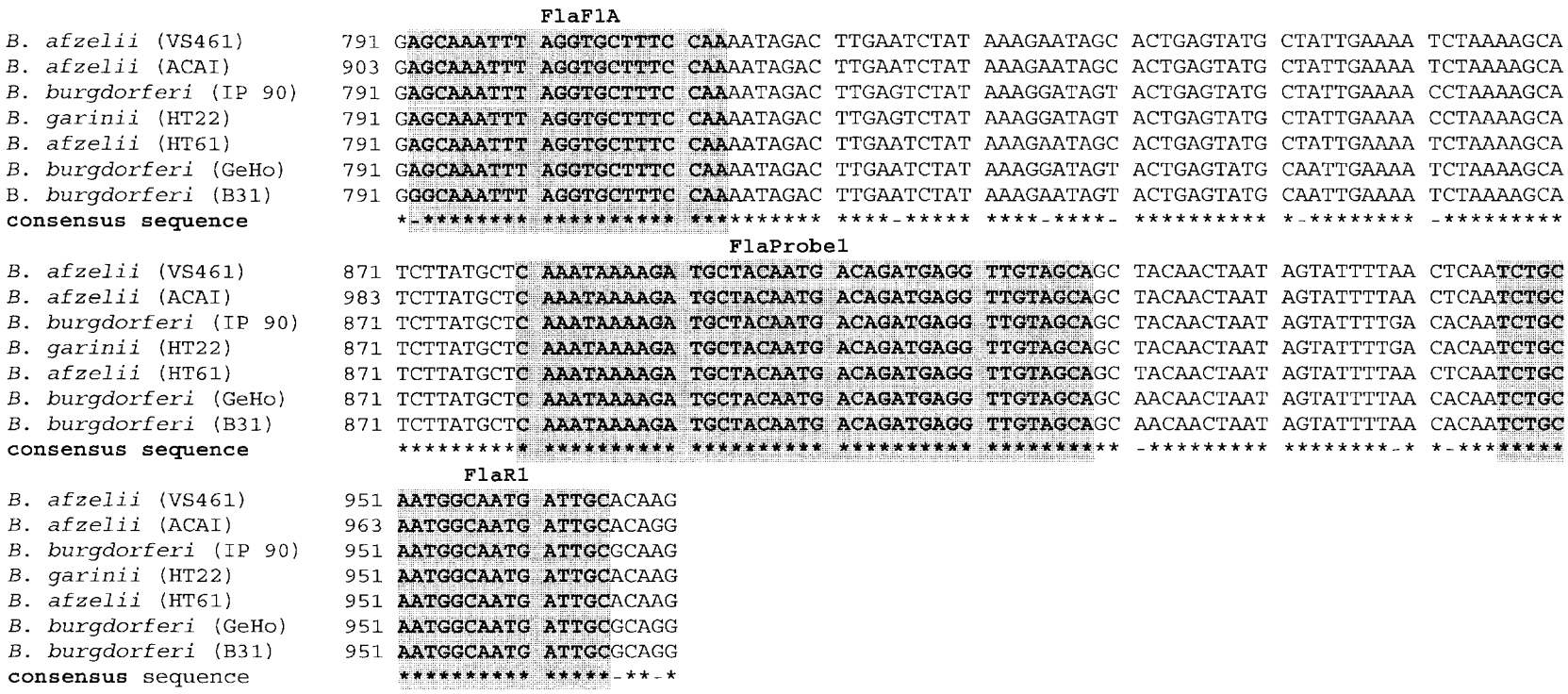

Figure 1 Multiple alignment of seven published flagellin gene sequences of *Borrelia burgdorferi* (sensu lato) strains. The nucleotides identical among all strains are indicated with asterisks in the consensus sequence. The forward primer FlaF1A, the reverse primer FlaR1 and the TaqMan probe FlaProbe 1 are printed in bold. The accession numbers (NCBI) of the flagellin gene sequences used for the comparison are: D63365 (*Borrelia afzellii* VS461), X75202 (*Borrelia afzelii* ACAI), L42885 (*Borrelia burgdorferi* IP90), D63367 (*Borrelia garinii* HT22), D63366 (*Borrelia afzelii* HT61), X15660 (*Borrelia burgdorferi* GeHo), and X15661 (*Borrelia burgdorferi* B31).

Table 1 Primers and the probe within the flagellin gene of *Borrelia burgdorferi* (sensu lato)

	Sequence (5′→3′)	Genome position[a]
Forward primer FlaF1A	AGC AAA TTT AGG TGC TTT CCA A	792–813
Reverse primer FlaR1	GCA ATC ATT GCC ATT GCA GA	965–946
Probe FlaProbe1	TGC TAC AAC CTC ATC TGT CAT TGT AGC ATC TTT TAT TTG	918–880

[a] Flagellin gene sequence of *Borrelia burgdorferi* (GeHo) (×15660).

Borrelia burgdorferi quantification standard

The plasmid pB31/41-9 (a gift from Dr R. Wallich, Department of Applied Immunology, Heidelberg, Germany), containing the flagellin gene of *Borrelia burgdorferi* B31 (X16833), was used as quantification standard. The concentration of the plasmid pB31/41-9 was measured by UV spectrophotometry. The plasmid stock was aliquoted at 1 μg/μL and stored at −20 °C. The aliquots were used only once for a serial dilution and generation of the standard curve.

TaqMan quantitative PCR assay

Preparation of the PCR mixture, extraction and pipetting of the clinical samples and amplification were done in three strictly separated laboratories, using dedicated laboratory equipment such as positive displacement pipettes, RNase- and DNase-free pipette tips and sterile surgical gloves.

The 50-μL PCR mixture consists of 25 μL of 2 × TaqMan Universal Mastermix, 300 nM FlaF1A forward primer, 900 nM FlaR1 reverse primer, 200 nM TaqMan probe, 5 μL of H_2O and 10 μL of the extracted target DNA. Each specimen was tested in duplicate. As amplification control and to test for the potential presence of inhibitors, an aliquot of the extract was also spiked with 1000 copies of the plasmid pB31/41-9. As extraction control, 1 × Tris-EDTA buffer (TE buffer) spiked with 500 copies of pB31/41-9 was extracted in parallel with the clinical specimens. Negative controls containing all PCR reaction components with the exception of template DNA (denoted no template control; NTC) were included after each third sample. In order to check for contamination with potential target DNA, two additional NTCs were pipetted in each of the pre-PCR laboratory rooms. Following an incubation step at 50 °C for 2 min (carryover prevention step, activation of the enzyme AmpErase (uracil-*N*-glycosylase, UNG)) and 10 min of incubation at 95 °C (denaturation of AmpErase UNG, activation of AmpliTaq Gold Polymerase), the samples were submitted to amplification (95 °C for 15 s, 60 °C for 1 min, 45 cycles) in an ABI Prism 7700 Sequence Detection System using optical tubes and caps.

Following amplification and real-time data acquisition, analysis was performed with a Macintosh computer 4400/200, applying the Sequence Detection System software version 1.6.3.

Nucleolytic cleavage of the TaqMan fluorogenic probe during PCR generates an increase of fluorescence that is proportional to the amount of the PCR product present in the reaction and may be calibrated by inclusion of serially diluted reference samples with known target DNA copy numbers. The threshold cycle (C_T) is defined as the cycle number at which the reporter fluorescence generated by cleavage of the probe passes a threshold set at 10 times the standard deviation of the mean baseline from cycle 3 to cycle 15.

RESULTS

Analytic sensitivity of the TaqMan PCR assay

The standard curve was generated with serial dilutions from 10 to 100 000 copies of the reference plasmid pB31/41-9, which contains the flagellin gene sequence of *Borrelia burgdorferi* sensu stricto (B31). A typical standard curve with a linear dynamic range of at least four orders of magnitude is shown in Figure 2. The correlation coefficient was routinely between 0.97 and 1.00.

Using plasmid dilutions as unknown specimens, a detection limit below 10 plasmid copies per assay was regularly obtained.

Reproducibility of the assay

To determine the intra-assay variation, identical series of 10 assays containing 50, 500 or 5000 copies of the standard plasmid pB31/41-9 were tested. The resulting coefficients of variation (in percentages) were 2.36, 1.77 and 0.78 for 50, 500 and 5000 copies, respectively (Table 2). The run-to-run precision (inter-assay variability) was tested by determination of identical copy numbers in 10 different PCR assays performed on different days by different people. The corresponding coefficients of variation (in percentages) were 3.47, 2.65 and 2.43 for 50, 500 and 5000 copies, respectively (Table 2).

Specificity of the TaqMan assay for strains of the taxon *Borrelia*

The specificity of the assay was tested with cultures from 48 isolates of the *Borrelia burgdorferi* complex (Table 3).

Among them, 37 are associated with Lyme disease: 12 *Borrelia burgdorferi* sensu stricto, 12 *Borrelia afzelii* and 13 *Borrelia garinii*.

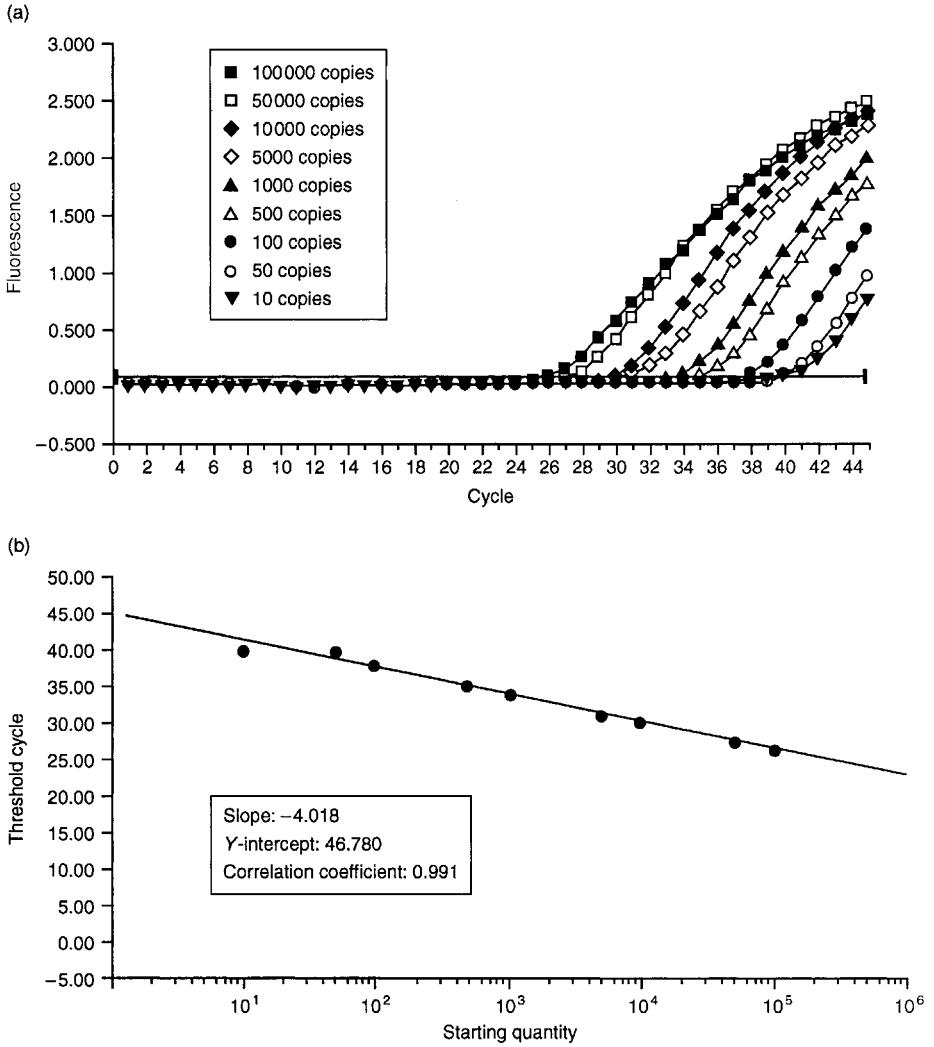

Figure 2 Amplification plot (a) and standard curve (b) of the *Borrelia burgdorferi* quantitative assay.

Eleven isolates of the species *Borrelia valaisiana*, whose clinical significance is still unresolved, were also tested.

Each of these 48 isolates was detectable (Table 3). Similar amplification rates were observed for the different species (*Borrelia burgdorferi* sensu stricto IP3, *Borrelia afzelii* VS461, *Borrelia garinii* VS102, *Borrelia valaisiana* VS 116), when using identical amounts of extracted DNA as template (data not shown).

Table 2 Coefficient of variation (CV) (in percentage) of precision within run and precision from run to run

	Standard DNA copies		
	50	**500**	**5000**
CV within run	2.36	1.77	0.78
CV from run to run	3.47	2.65	2.43

In order to further evaluate the specificity of the assay, 10 additional *Borrelia* strains (*Borrelia andersonii* (19952), *Borrelia bissettii* (DN 127), *Borrelia anserina*, *Borrelia coriaceae*, *Borrelia hermsii*, *Borrelia japonica* (Fi340), *Borrelia parkeri*, *Borrelia turicatae* and two strains of the group *Borrelia lusitaniae* (IR345, BR41)) were tested under standard conditions using culture material as substrate. In contrast to the 48 *Borrelia burgdorferi* strains, no or only background amplification signals were recorded for species other than *Borrelia burgdorferi* sensu lato (data not shown).

The possibility of cross-reactions with unrelated pathogens which may be found in clinical specimens was assessed using various bacteria, viruses and fungi. No signal was recorded with *Escherichia* sp., *Salmonella* Enteritidis, *Acinetobacter* sp., *Proteus vulgaris*, *Escherichia coli* O157, *Neisseria meningitidis*, *Pseudomonas*

Table 3 Specificity of the TaqMan assay tested with 48 isolates of the taxon *Borrelia*

Species	Isolates	Countries	Biological origin
Borrelia afzelii	VS461	Switzerland	*Ixodes ricinus*
	P/Sto	Germany	Human (skin)
	Pspe	Germany	Human (CSF)
	DK3	Denmark	Human (skin)
	Iper	Japan	*Ixodes persulcatus*
	A100S	Netherlands	Human (skin)
	M55	Netherlands	*Ixodes ricinus*
	ACA1	Sweden	Human (skin)
	UMO1	Sweden	Human (skin)
	VS18	Switzerland	*Ixodes ricinus*
	VS25R-OR	Switzerland	*Apodemus flavicollis*
	VS42R-R	Switzerland	*Apodemus sylvaticus*
Borrelia burgdorferi	B31	USA	*Ixodes daminii*
	Geho	Germany	Human (skin)
	IP3	France	Human (CSF)
	IP1	France	Human (CSF)
	BE1	Switzerland	Human (synovial fluid)
	VS14	Switzerland	*Ixodes ricinus*
	VS109	Switzerland	*Ixodes ricinus*
	VS206	Switzerland	*Ixodes ricinus*
	VS423	Switzerland	*Ixodes ricinus*
	VS753	Switzerland	*Ixodes ricinus*
	MAC3EMCNY86	USA	Human (skin)
	13062	Yugoslavia	*Ixodes ricinus*
Borrelia garinii	20047	France	*Ixodes ricinus*
	387	Germany	Human (CSF)
	P/Bi	Germany	Human (CSF)
	FAR02	Denmark	*Ixodes uriae*
	BITS	Italy	*Ixodes ricinus*
	A77C	The Netherlands	Human (CSF)
	VS102	Switzerland	*Ixodes ricinus*
	VS307	Switzerland	*Ixodes ricinus*
	VS468	Switzerland	*Ixodes ricinus*
	VSBP	Switzerland	Human (CSF)
	VSDA	Switzerland	Human (CSF)
	NBS16	Sweden	*Ixodes ricinus*
	Ip89	CIS	*Ixodes persulcatus*
Borrelia valaisiana	VS116	Switzerland	*Ixodes ricinus*
	F10.8.94	Germany	*Ixodes ricinus*
	Frank	Germany	*Ixodes ricinus*
	Z6.11.93	Germany	*Ixodes ricinus*
	UK	UK	*Ixodes ricinus*
	AR-2	The Netherlands	*Ixodes ricinus*
	M19	The Netherlands	*Ixodes ricinus*
	M52	The Netherlands	*Ixodes ricinus*
	M57	The Netherlands	*Ixodes ricinus*
	AG1	Switzerland	*Ixodes ricinus*
	VS732	Switzerland	*Ixodes ricinus*

aeruginosa, Streptococcus pneumoniae, Enterococcus sp., *Streptococcus agalactiae*, *Listeria monocytogenes*, *Bacillus subtilis, Staphylococcus saprophyticus*, *Candida* sp., hepatitis B virus, adenovirus, human parvovirus B19, herpes simplex virus type 1, and herpes simplex virus type 2.

Clinical samples

Sensitivity of the TaqMan assay in clinical samples

When testing synovial fluid, CSF, blood and synovial membrane biopsies spiked with the plasmid pB31/41-9 prior to amplifica-

Table 4 Detection and quantitation of *Borrelia burgdorferi* (sensu lato) DNA in patients and controls

Study group	Specimen	Number of patients	Number of samples	Positive (%)	Amount DNA[a]	Negative (%)
A	CSF	54	56	1 (1.8)	63	55 (98.2)
B	Synovial fluid	27	28	5 (17.9)	41 000	23 (82.1)
					25 300	
					5800	
					100	
					20	
	Synovial biopsies	4	5	1 (20)	70	4 (80)
C	Blood	39	39	0 (0)		39 (100)
	Synovial fluid	7	7	0 (0)		7 (100)
	CSF	33	34	0 (0)		34 (100)
	Synovial biopsies	3	3	0 (0)		3 (100)
	Urine	1	1	0 (0)		1 (100)

A, patients with clinical suspicion of neuroborreliosis; B, patients with unclear arthritis; C, negative controls (blood donors and patients without clinical suspicion of Lyme disease).
[a]Number of DNA copies per milliliter or biopsy.

tion, a detection limit below 10 copies was regularly achieved (data not shown).

In order to evaluate the suitability of the *Borrelia burgdorferi* TaqMan assay for routine use, we tested for the presence of *Borrelia burgdorferi* sensu lato DNA in 173 samples from 168 patients grouped into three categories as follows (Table 4).

Patients with neurologic symptoms

Of 56 CSF samples taken from 54 patients with clinical suspicion of neuroborreliosis, only one (1.8%) tested positive with 63 copies of *Borrelia burgdorferi* DNA/mL CSF (Table 4). A corresponding serum sample gave a negative result when tested by PCR (data not shown).

Patients with clinical suspicion of Lyme arthritis

Among 28 synovial fluid specimens from 27 patients, five (17.9%) contained *Borrelia burgdorferi* sensu lato DNA, as well as one (20%) of five synovial membrane biopsies from four patients (Table 4). Whereas 20–41 000 genome equivalents were detected in the synovial fluid samples, 70 genome equivalents were counted in the biopsy (Table 4). No *Borrelia burgdorferi* DNA was detected in blood samples available for five of six patients who had the bacterial genome detected in synovial fluid or synovial membrane.

The presence of inhibitors of the *Taq* DNA polymerase was assessed by spiking aliquots of 89 samples (groups A and B) with 1000 copies of the plasmid pB31/41-9 prior to amplification. The reaction was inhibited in three (3.4%) samples. However, the inhibitory effect was overcome by a two-fold dilution of the extracts prior to amplification.

Negative control group

In order to test for the absence of false-positive results, 37 serum samples from 37 blood donors without evidence of Lyme disease were investigated. None of the samples tested in duplicate showed measurable amounts of *Borrelia burgdorferi* DNA.

In addition, one urine sample, 34 CSF samples, seven synovial fluid samples, two blood samples and three synovial membrane biopsies from 46 patients with unrelated infections were also tested. *Borrelia burgdorferi* DNA could not be detected in any of these samples (Table 4). The presence of inhibitors of the enzyme *Taq* DNA polymerase in these samples was assessed by spiking corresponding aliquots with 1000 copies of the standard plasmid. None of the reactions was inhibited.

DISCUSSION

The significance of PCR-based assays to complement the clinical or serologic diagnosis of Lyme disease has so far been widely documented [1,10–21]. However, the majority of the published PCR protocols are not suitable for day-to-day testing in a routine laboratory setting. Namely, complex extraction and detection protocols or technically challenging nested-PCR formats have often been used to ensure maximal sensitivity. Moreover, contamination and carryover prevention measures to minimize the risk of false-positive results or the use of internal controls to monitor the possible occurrence of false-negative results have not been systematically included. In addition, most of the protocols published are not designed for quantitative detection of *Borrelia burgdorferi* DNA. Quantitation of the bacterial load in clinical samples may be helpful to monitor treatment efficacy, to gain some insight into the pathogenesis of the infection, and to determine whether a correlation does exist between clinical symptoms and the amount of bacteria detected. Quantitative detection of *Borrelia burgdorferi* DNA has been recently documented in mouse tissue samples using the LightCycler technology and SYBR green as double-

stranded DNA dye [22] or a microtiter-based competitive PCR assay [23]. A real-time PCR assay for the quantitative detection of *Borrelia burgdorferi* has been recently reported by Pahl et al. [24]. These authors used a mouse model of Lyme disease to evaluate the usefulness of their assay for the detection of *Borrelia burgdorferi* DNA in tissue samples. However, the system was not evaluated in a routine diagnostic setting with human clinical specimens. TaqMan PCR has also been used to detect *Borrelia burgdorferi* DNA in ticks [25] and in canine skin punch biopsy and blood samples [26].

Taking into account the specific diagnostic parameters and demands of a clinical laboratory setting, we have developed a quantitative TaqMan PCR assay which allows the detection of the clinically relevant strains within the *Borrelia burgdorferi* sensu lato complex. In contrast to other protocols published previously, the ability of our assay to detect *Borrelia burgdorferi* sensu lato was documented with the successful amplification of a large number of strains. Namely, each one of the 12 *Borrelia burgdorferi* sensu stricto, 12 *Borrelia afzelii* and 13 *Borrelia garinii* isolates investigated tested positive. In addition, 11 *Borrelia valaisiana* isolates were also detected with the same sensitivity. This observation is of particular interest, because *Borrelia valaisiana* (group VS116) has previously been detected in skin biopsies of patients with EM [1], and the possible involvement of *Borrelia valaisiana* has recently been suggested for patients presenting with chronic clinical manifestations [2]. The ability of our test to detect *Borrelia valaisiana* may therefore aid in the investigation of the clinical role of this novel species.

The simple and reliable DNA extraction method combined with the highly sensitive PCR assay and the absence of post-amplification steps allowed us to perform the assay and to report the results within one working day. Hence, the main features of the assay format, such as robustness, speed and convenience, were of high utility in routine diagnosis. In addition, the precision and reproducibility of the assay may become important factors in monitoring disease progression and therapeutic efficacy, particularly in patients with chronic disease, when the measurement of bacterial load in multiple follow-up samples requires a constant baseline. The choice of a closed-tube system, together with the use of the enzyme uracil-*N*-glycosylase and strict dedication of equipment, efficiently contributed to the avoidance of any detectable contamination or carryover during an 8-month evaluation of the test under routine conditions (data not shown). Hence, the risk of contamination leading to false-positive PCR results, which is a major problem associated with other PCR assay formats, is very well controlled in our case. In addition, the presented format included monitoring for the potential presence of inhibitors possibly causing false-negative results, a major advantage in achieving sound validity of diagnostic results.

The applicability of the TaqMan assay to detect *Borrelia burgdorferi* DNA in a routine setting was assessed with unselected clinical samples sent to our diagnostic laboratory. These samples were not obtained from a population of patients with confirmed Lyme disease but from patients with anamnestic or clinical suspicion of infection with *Borrelia burgdorferi* only. Of the 56 CSF samples from 54 patients with neurologic symptoms, only one (1.8%) tested positive. A possible explanation for this low positive rate might be very low levels of organisms present [13,21] and/or the small volumes of CSF generally available for the extraction. Thus, and despite its high sensitivity, PCR analysis of CSF may not be useful to substantiate a clinical suspicion of neuroborreliosis. Indeed, in a study where none of 471 CSF specimens submitted for *Borrelia burgdorferi* detection were PCR positive, Tang et al. [27] recently suggested that PCR of CSF is not suitable as screening test for the diagnosis of Lyme neuroborreliosis.

In contrast, the detection of *Borrelia burgdorferi* DNA in synovial fluid and in synovial membrane specimens obtained from patients with rheumatologic manifestations illustrates the usefulness of our PCR assay to confirm the diagnosis of Lyme arthritis. Quantitative measurement of the bacterial burden might be particularly useful in the case of patients with persistent or chronic infections [12,24,28]. Namely, detection of *Borrelia burgdorferi* DNA in the synovial fluid has been reported in a 12-year-old patient with an onset of Lyme arthritis 5 years after EM [29]. In addition, the quantitative PCR assay may help us to monitor the elimination of *Borrelia burgdorferi* DNA following antibiotic treatment. This might, for instance, be useful for patients with treatment-resistant Lyme arthritis who may have intra-articular persistence of *Borrelia burgdorferi* DNA in synovial membrane [30]. However, PCR results for *Borrelia burgdorferi* DNA may be negative in synovial specimens obtained from patients with antibiotic treatment-resistant Lyme arthritis and persistent synovial inflammation [31].

Specific clinical studies aimed at establishing the diagnostic significance of the present real-time PCR assay among conventional laboratory diagnostic tools need to be conducted.

In summary, we have established a TaqMan PCR assay for the quantitative detection of *Borrelia burgdorferi* sensu lato DNA in routine laboratory practice. This assay may become a valuable tool to complement the standard serology and to strengthen the diagnosis of Lyme disease. In addition, the quantitative TaqMan PCR assay for *Borrelia burgdorferi sensu* lato may prove to be useful to monitor disease progression and response to antibiotic treatment.

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